

## INFLUENCE OF FORMULATION FACTORS ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF DERMAL MICROEMULSIONS PREPARED WITH SUCROSE ESTERS

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### Abstract

The objective of the present paper was to study the influence of some formulation factors – such as the surfactant: co-surfactant ratio and the proportion of hydrophilic: lipophilic phase - on the conditions of formulation, preparation and physico-chemical characteristics of avocado oil dermal microemulsions obtained with sucrose esters. In the first phase of the study we prepared three series of formulations where the proportion of surfactant: co-surfactant (sucrose laurate: Transcutol) (SL: TC) varied as follows: 1: 1, 1: 3 and 3: 1, respectively. In the second phase of the study we selected and prepared five microemulsion formulations labeled F1 - F5, in which the SL:TC (1: 1) percentage was constant, along the dilution line 70, while the hydrophilic: lipophilic phase ratio varied in the following proportions: 1: 5, 1: 2, 1: 1, 2: 1 and 5: 1, respectively. The microemulsions were characterized by the determination of the following parameters: electrical conductivity, rheological behaviour, pH, transepidermal water loss coefficient. The data obtained demonstrated that: the analyzed formulations belong to the Newtonian fluid flow category; electrical conductivity increased directly proportional to the percentage of hydrophilic phase in the formula and the pH was in the near-physiological range; F3 - F5 formulations containing > 15% hydrophilic phase in the formulation had a strong moisturizing effect. The microemulsions were well tolerated on the skin and no irritant effect was recorded.

### Rezumat

Obiectivul acestei cercetări a constat în studiul influenței unor factori de formulare (raportul surfactant: co-surfactant și proporția fază hidrofilă: fază lipofilă) asupra condițiilor de formulare, preparării și caracteristicilor fizico-chimice ale unor dermomicroemulsii cu ulei de avocado preparate cu esteri ai sucrozei. În prima etapă a acestui studiu au fost preparate trei serii de formulări în care raportul surfactant: co-surfactant (sucroză laurat: Transcutol) (SL: TC) a fost de 1: 1, 1: 3 și respectiv 3: 1. În etapa a doua a cercetării am selectat și preparat cinci formulări de microemulsii notate F1 – F5, în care procentul SL: TC (1: 1) a fost constant pe linia de diluție 70, în timp ce raportul fază hidrofilă: fază lipofilă a variat după cum urmează: 1: 5, 1: 2, 1: 1, 2: 1 și 5: 1. Microemulsiile au fost caracterizate prin determinarea următorilor parametri: conductivitate electrică, comportament reologic, pH, coeficient de pierdere transepidermică de apă. Conform rezultatelor obținute apreciem că: formulările studiate se încadrează în categoria fluidelor Newtoniene; conductivitatea electrică a crescut direct proporțional cu procentul de fază hidrofilă din formulă; valoarea pH-ului a fost apropiată de pH-ul fiziologic;

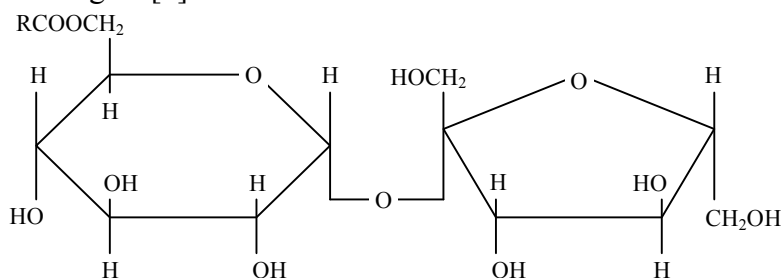
formulările F3-F5 cu un conținut de fază hidrofilă > 15% au exercitat un efect hidratant puternic. Microemulsiile au fost bine tolerate pe piele și nu s-a observat niciun efect iritant.

**Keywords:** microemulsions, sucrose esters, avocado oil.

### Introduction

Although microemulsions have numerous advantages as drug delivery systems, these modern pharmaceutical formulations have a limited applicability in the pharmaceutical and cosmetic field due to the quality conditions imposed by current legislation, which raw materials must meet. These limitations particularly concern the surfactants and cosurfactants present in a high percentage in the formulation of microemulsions. Synthetic surfactants such as sodium lauryl sulphate, Tweens, Spans, different sorts of Brij, quaternary ammonium salts, etc., as well as some synthetic cosurfactants, have an irritant and allergen effect on the skin [1, 14]. In this context, the selection of substances used in the preparation of microemulsions for pharmaceutical and cosmetic use increasingly concerns researchers. For the past years there has been a growing interest for the formulation of microemulsions with sucrose esters as surfactants; this pharmaceutical form has become an alternative to conventional macroemulsions and other drug delivery systems administrated by various routes [8, 10, 18, 19]. Sucrose esters are non-ionic, biodegradable, biocompatible and non-irritating surfactants, whose molecule is composed of a hydrophilic part (sucrose) and a hydrophobic part (fatty acid radical: lauric, palmitic, stearic, oleic acid) (Figure 1) [17].

The phase behaviour of sucrose esters is much less influenced by temperature than the phase behaviour of other non-ionic surfactants (i.e. ethylene oxide based surfactants). Sucrose esters are versatile surfactants whose hydrophilic and lipophilic properties can be adjusted by varying fatty acid chain lengths [1].



**Figure 1**  
Sucrose esters - general structure  
(R – the hydrophobic part)

In the research presented in this paper we used sucrose-laurate (SL), a surfactant with a hydrophilic-lipophilic balance value of 16, for which a research study was performed on its use in some dermocosmetic formulations [2, 5, 16]. Transcutol<sup>®</sup> as a co-surfactant was studied in the selected formulations [9]. The selection of the oily phase is of major importance in the formulation of microemulsions, because it will influence the selection of the other microemulsion components (mainly for O/W type microemulsions) [13]. It is known that oils with excessively long chains (or with a high molecular weight) such as soybean oil are difficult to be microemulsified, while short-chain oils (or with a low molecular weight) such as mono-, di -and medium chain triglycerides are more easily microemulsified [3, 4, 11, 12]. In our research, the lipophilic phase of the microemulsion was the avocado oil obtained by cold-press extraction from the avocado fruit (*Persea gratissima*, *Lauraceae*). Avocado oil has a high content of saturated and unsaturated fatty acids, along with liposoluble vitamins, minerals and oligoelements (Table I) [15]. Due to its chemical composition, avocado oil is involved in maintaining the skin barrier function, has nutritional action on skin tissue and reduces the intensity of skin desquamation [7].

**Table I**  
Content of fatty acids and vitamins of avocado oil

| Fatty acids                                | Average content of fatty acids (g%) | Vitamins                           | Average content of vitamins (mg%) |
|--------------------------------------------|-------------------------------------|------------------------------------|-----------------------------------|
| <b>Saturated</b>                           |                                     | Carotenes (pro vitamin A)          | 0.13 → 0.51                       |
| Myristic                                   | 0.47                                | Thiamine(B <sub>1</sub> )          | 0.08 → 0.12                       |
| Palmitic                                   | 15.80                               | Riboflavin (B <sub>2</sub> )       | 0.21 → 0.23                       |
| Stearic                                    | 0.58                                | Pyridoxine (B <sub>6</sub> )       | 0.45                              |
| <b>Monounsaturated</b>                     |                                     | Niacin (B <sub>3</sub> )           | 1.45 → 2.16                       |
| Palmitoleic                                | 4.15                                | Pantothenic acid (B <sub>5</sub> ) | 0.90 → 1.14                       |
| Oleic                                      | 49.50                               | Folic Acid (B <sub>9</sub> )       | 0.018 → 0.040                     |
| <b>Polyunsaturated</b>                     |                                     | Biotin (B <sub>7</sub> )           | 0.003 → 0.006                     |
| Linoleic                                   | 25.10                               | Ascorbic acid                      | 13.0 → 37.0                       |
| Linolenic                                  | 3.59                                | Calciferol                         | 0.01                              |
| Arachidonic                                | 0.61                                | $\alpha$ – tocopherol              | 3.0                               |
| <b>Total saturated fatty acids (SFA)</b>   | <b>16.85</b>                        | 2- methyl-1,4-naphthoquinone       | 0.008                             |
| <b>Total unsaturated fatty acids (UFA)</b> | <b>78.95</b>                        | -                                  | -                                 |
| <b>UFA/SFA ratio</b>                       | <b>4.68</b>                         | -                                  | -                                 |

Our objective was to study the influence of some formulation factors (the surfactant: cosurfactant ratio and hydrophilic: lipophilic phase proportion) on the formulation conditions, preparation and physico-

chemical characterization of some avocado oil microemulsions. In this preliminary study of the formulation of avocado oil microemulsions based on sucrose esters, we aimed to understand the physico-chemical states of the prepared microemulsions, considering that in the literature there are few research studies on the preparation of microemulsions with vegetable oils.

#### **Materials and Methods**

*Sucrose laurate* containing > 80% mono-, bi and -triesters was kindly donated by Mitsubishi Chemical Europe GmbH (Duesseldorf, Germany); *monoethylether diethylene glycol* (Transcutol ®) (Gattefosse, Lyon, France), *avocado oil* (Natural Sourcing LLC, Oxford, England). When preparing the microemulsions we used *purified water* and other reagents of a suitable quality, according to the requirements of Romanian Pharmacopoeia 10<sup>th</sup> edition.

#### **The determination of the ternary phase diagram and the preparation of microemulsions**

In order to identify the appropriate concentration domain of the microemulsion forming area, ternary phase diagrams were designed using the water titration method at room temperature as described by M. Fanun [6]. Three series of formulations were prepared, in which the proportion of surfactant: co-surfactant (SL-TC) varied as following: 1:1, 1:3 and 3:1 respectively. In the last stage of the study we selected and prepared five formulations of some microemulsions, labeled F1 - F5, in which the ratio SL:TC (1:1) was constant on the dilution line 70 (this means that the SL:TC (1:1) ratio is 70% of the total weight of the formulation), while the hydrophilic: lipophilic phase ratio varied in the following proportions: 1:5, 1:2, 1:1, 2:1 and 5:1 respectively.

**The physico-chemical characterization of microemulsions** consisted in the evaluation of the following parameters: **Electrical conductivity** ( $\sigma$ ) was determined using Metrhom 712 conductometer (Herisau, Switzerland) with graphite electrode at room temperature ( $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ). The conductometric cell was calibrated with standard KCl solution. **Rheological behaviour - the dynamic viscosity** ( $\eta$ ) was determined by rotational rheoviscometer Rheolab MC120 (Stuttgart, Germany). **pH measurement** - was performed using Thermo Orion pH meter (Thermo Fisher, Florida, USA). All measurements were performed three times at room temperature ( $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) and the presented results represent the average value of the three determinations ( $\pm$  SD).

**The evaluation of transepidermal water loss** was performed using the Tewa-meter TM 210 (Courage + Khazaka, Germany) on groups of six volunteers, aged between 20-35 years, after their informed consent was

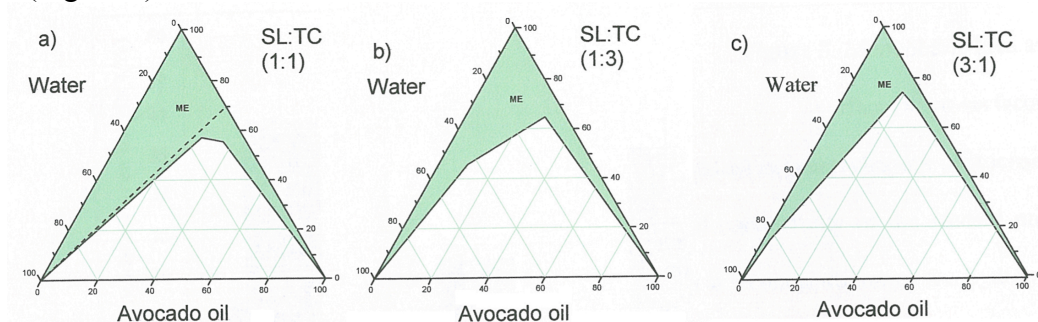
signed. The transepidermal water loss coefficient was measured immediately after the application of the microemulsion on the back of the palm (an area exposed to air, with a low density of sebaceous glands), and it was considered the zero time of determinations, followed by successive measurements every 30 minutes for two hours. The experiment was performed in three consecutive days and results are expressed as the average of the three determinations.

Following the determination of transepidermal water loss coefficient we also performed a visual assessment of the irritating potential of the studied formulations. The tolerability of the microemulsion formulations was reported and interpreted according to the COLIPA (The European Cosmetic Association) scores, which provides standardized values for **erithema** (0 = no evidence of erithema; 0.5 = minimal or doubtful erithema; 1 = slight redness, spotty and diffuse; 2 = moderate, uniform redness; 3 = strong uniform redness; 4 = fiery redness) **dryness** (0 = no evidence of scaling; 0.5 = dry without scaling; 1 = fine/mild scaling; 2 = moderate scaling; 3 = severe scaling with large flakes) and **oedema** (- = absence of oedema; + = presence of oedema) [20].

## Results and Discussion

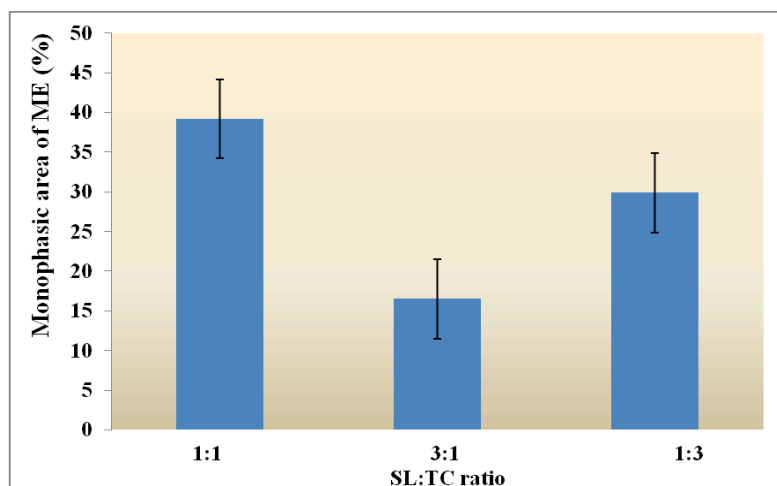
### Analysis of ternary phase diagrams

According to the obtained results, the surfactant: co-surfactant ratio had a major influence on the behaviour of microemulsion phases. From the analysis of the diagram corresponding to each report (Figure 2. a, b and c) it can be observed that the formulations containing a 1:1 surfactant: co-surfactant ratio have the largest microemulsion area. The smallest single-phase region was obtained for the formulations with SL:TC in a ratio of 3:1 (Figure 3).



**Figure 2**

Ternary phase diagrams of systems SL / TC / avocado oil / water  
(a. SL / TC = 1:1; b. SL / TC = 1:3; c. SL / TC = 3:1)

**Figure 3**

Monophasic area according to the  
surfactant: co-surfactant ratio (ME - microemulsions)

We noticed that the presence of cosurfactant caused a decrease in interfacial tension and promotes the formation of microemulsions by increasing SL solubility in the aqueous phase. In the absence of the cosurfactant, it is more difficult to obtain microemulsions because SL is poorly soluble in the oil phase, as revealed by other data in literature [4, 5].

#### Physico-chemical characterization of microemulsions

The electrical conductivity of the studied microemulsions increased directly proportionally with the concentration of the hydrophilic phase in the system (Table II).

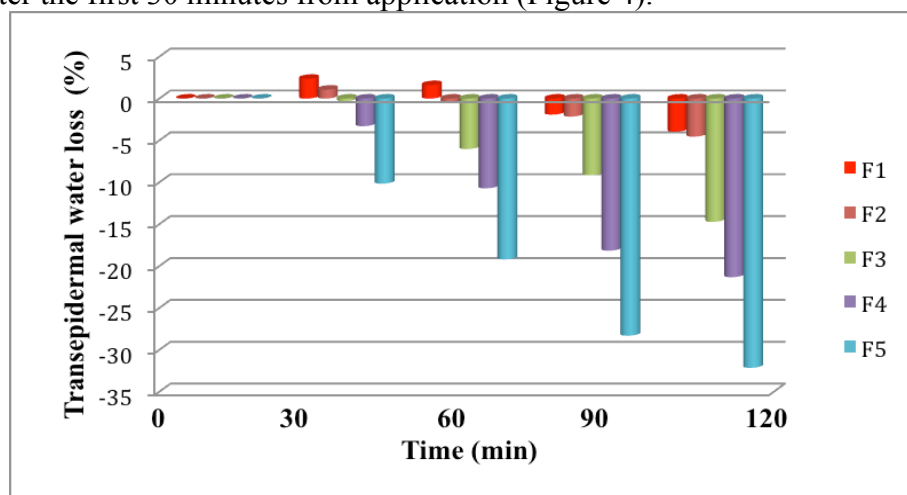
**Table II**

Physico - chemical parameters of microemulsions with avocado oil

| Formulation | Concentration of the hydrophylic phase (% w/w)<br>[hydrophilic: lipophilic phase ratio] | $\sigma$<br>Electrical conductivity (mS/cm) ( $\pm$ SD) | $\eta$<br>Viscosity (mPa·s) ( $\pm$ SD) | pH ( $\pm$ SD)     |
|-------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------|-----------------------------------------|--------------------|
| F1          | 5.00<br>[1:5]                                                                           | 1.98 ( $\pm$ 1.21)                                      | 140.25 ( $\pm$ 0.87)                    | 5.02 ( $\pm$ 0.84) |
| F2          | 10.00<br>[1:2]                                                                          | 3.86 ( $\pm$ 1.08)                                      | 146.11 ( $\pm$ 2.01)                    | 5.19 ( $\pm$ 0.54) |
| F3          | 15.00<br>[1:1]                                                                          | 12.51 ( $\pm$ 0.94)                                     | 153.32 ( $\pm$ 1.58)                    | 5.21 ( $\pm$ 0.60) |
| F4          | 20.00<br>[2:1]                                                                          | 23.13 ( $\pm$ 1.62)                                     | 166.15 ( $\pm$ 1.31)                    | 5.38 ( $\pm$ 0.47) |
| F5          | 25.00<br>[5:1]                                                                          | 40.50 ( $\pm$ 1.42)                                     | 180.50 ( $\pm$ 1.74)                    | 5.80 ( $\pm$ 0.63) |

The dynamic viscosity values of the studied microemulsions were in the range 140-180 mPa·s, which proves that all formulations presented a Newtonian behaviour. The pH, an important parameter for the formulations with topical application, showed values close to the physiological pH.

For the transepidermal water loss coefficient there were recorded significantly lower values for the formulations with a high content of water after the first 30 minutes from application (Figure 4).



**Figure 4**

Values of transepidermal water loss coefficient of microemulsions with avocado oil

A proportion of over 15% hydrophilic phase in the formulation led to a strong moisturizing effect.

In terms of **irritating potential**, the studied formulations were evaluated with Colipa score 0 for erythema and dryness or scaling and – for oedema. Basically, we have not observed any irritation, drying, redness or swelling phenomena on the skin areas where we applied the microemulsion formulations with avocado oil based on esters of sucrose. These results suggest a good skin tolerance of the studied formulations, although they had an increased surfactant content.

## Conclusions

According to the ternary phase diagram, a SL: TC ratio of 1:1 led to the most extensive range of single-phase system, corresponding to microemulsions. In this area, on line 70 of the SL:TC system, five formulations were selected in which we varied the hydrophilic: lipophilic phase ratio as following: 1:5, 1:2, 1:1, 2:1 and 5:1 respectively. The results

obtained from the physico-chemical characterization of these microemulsions showed that the analyzed formulations fall within the Newtonian fluid flow, that electrical conductivity exhibited an increase in direct proportion to the percentage of hydrophilic phase in the formulation and that the pH was in the near-physiological range. F3 - F5 formulations containing more than 15% hydrophilic phase in the formulation had a strong moisturizing effect after 30 mins from application. In addition, microemulsions were well tolerated on the skin and no irritant effect was recorded. According to the preliminary results obtained in these studies, we consider that in the future the studied formulations can become systems of topical active substances administration as dermocosmetic products. In addition, these formulations will be investigated further in order to elucidate the internal structure of microemulsions and to evaluate the incorporation capacity of drug substances by sophisticated physical techniques, such as: small angle X-ray scattering, small angle neutron scattering, transmission electron microscopy and nuclear magnetic resonance.

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